Fluorescent tagged episomals for stoichiometric induced pluripotent stem cell reprogramming.

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Public Summary:
Non-integrating episomal vectors have become an important tool for induced pluripotent stem cell (iPS) reprogramming. The episomal vectors carrying the Yamanaka reprogramming factors (Oct4, Sox2, Klf, and Myc) function similarly to retrovirus or lentiviral constructs, yet they are lost through cell division, resulting in pluripotent stem cells that maintain an unmodified genetic background. Given that induction of pluripotency is a highly stochastic process, tight regulation of reprogramming factor dosage, such as those available in viral systems may allow for more efficient generation of higher quality iPSCs. We present a modified set of vectors that express separable fluorescent proteins to allow for enrichment of transfected cells, and control of reprogramming factor copy number and relative dosage.

Scientific Abstract:
BACKGROUND: Non-integrating episomal vectors have become an important tool for induced pluripotent stem cell reprogramming. The episomal vectors carrying the "Yamanaka reprogramming factors" (Oct4, Klf, Sox2, and L-Myc + Lin28) are critical tools for non-integrating reprogramming of cells to a pluripotent state. However, the reprogramming process remains highly stochastic, and is hampered by an inability to easily identify clones that carry the episomal vectors. METHODS: We modified the original set of vectors to express spectrally separable fluorescent proteins to allow for enrichment of transfected cells. The vectors were then tested against the standard original vectors for reprogramming efficiency and for the ability to enrich for stoichiometric ratios of factors. RESULTS: The reengineered vectors allow for cell sorting based on reprogramming factor expression. We show that these vectors can assist in tracking episomal expression in individual cells and can select the reprogramming factor dosage. CONCLUSIONS: Together, these modified vectors are a useful tool for understanding the reprogramming process and improving induced pluripotent stem cell isolation efficiency.